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(54) Title
ULTRASONIC CONTRAST AGENTS, PROCESS FOR PRODUCING THEM AND THEIR USE AS DIAGNOSTIC AND THERAPEUTIC AGENTS

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(57) Claim

1. An ultrasonic contrast agent comprising of physiologically acceptable carrier liquid, and microparticles which contain a gas and/or a organic fluid with a boiling point below 60°C, said microparticles consisting of:
(a) one or more amyloses, or
(b) one or more synthetic biodegradable polymers.

14. Microparticles for use in ultrasonic contrast agents, said microparticles containing a gas and/or an organic fluid with a boiling point below 60°C and said microparticles consisting of:
(a) one or more amyloses, or
(b) one or more synthetic biodegradable polymers.

21. A process for the preparation of microparticles of synthetic biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16, or 18 characterised in that a polymer or copolymer is dissolved in one or more organic solvents which are not miscible with water, and is then emulsified in water, and the emulsion thus obtained is then filtered and dried.

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22. A process for the preparation of microparticles of synthetic biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16 or 18 characterised in that a polymer or copolymer is dissolved in one or more solvents containing gas bubbles and is then precipitated or emulsified in water, the suspension or emulsion obtained is then filtered off and dried.

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(54) Title: ULTRASONIC CONTRAST AGENTS, PROCESS FOR PRODUCING THEM AND THEIR USE AS DIAGNOSTIC AND THERAPEUTIC AGENTS

(54) Bezeichnung: ULTRASCHALLKONTRASTMITTEL, VERFAHREN ZU DEREN HERSTELLUNG UND DEREN VERWENDUNG ALS DIAGNOSTIKA UND THERAPEUTIKA

(57) Abstract

Ultrasonic contrast agents consisting of microparticles containing amyloses or synthetic biodegradable polymers and a gas and/or a liquid with a boiling point below 60°C, process for producing them and their use as diagnostic or therapeutic agents.

(57) Zusammenfassung

Die Erfindung betrifft Ultraschallkontrastmittel bestehend aus Mikropartikeln, die aus Amylosen oder synthetischen, bioabbaubaren Polymeren und einem Gas und/oder einer Flüssigkeit mit einem Siedepunkt unter 60°C bestehen, Verfahren zu deren Herstellung und deren Verwendung als Diagnostika und Therapeutika.

ULTRASONIC CONTRAST AGENTS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS A DIAGNOSTIC AND THERAPEUTIC AGENT

The invention relates to microparticles according to the preamble of Patent Claim 1, a process for their preparation and their use as a diagnostic and therapeutic agent.

It is known that cardial echo contrasts can be achieved through peripheral injection of solutions which contain fine gas bubbles (Roelandt J, Ultrasound Med Biol 8: 471-492, 1982). These gas bubbles are obtained in physiologically compatible solutions, eg through shaking, other agitation or through the addition of carbon dioxide. However they are not standardized in terms of number and size and cannot be adequately reproduced. Also they are as a rule not stabilized so that their service life is short. Their average diameters are generally above the erythrocyte size so that it is not possible to obtain pulmonary capillary passages with subsequent contrasting of organs such as the left heart, liver, kidneys or spleen. Furthermore they are not suitable for quantifications since the ultrasonic echo which they produce is made up from several processes which cannot be separated from each other such as the formation of the bubbles, coalescence and dissolution. Thus it is not possible for example to obtain definite details on the transit times with the aid of these ultrasonic contrast agents by measuring the contrast path in the myocardium. This requires contrast agents whose dispersal bodies are not subject to their own kinetics.

In addition there are ultrasonic contrast agents in the form of particles (Ophir, Gobuty, McWhirt, Maklad, Ultrasonic Backscatter from Contrast-producing Collagen Microspheres, Ultrasonic Imaging 2:66-67, 1980). Furthermore solutions of a higher density are used as ultrasonic contrast agents

(Ophir, McWhirt, Maklad, Aqueous Solutions as Potential Ultrasonic Contrast Agents, Ultrasonic Imaging 1:265-279, 1979 as well as Tyler, Ophir, Maklad, In-vivo Enhancement of Ultrasonic Image Luminance by Aqueous Solutions with High Speed of Sound, Ultrasonic Imaging 3:323-329, 1981). It is also known to use emulsions as ultrasonic contrast agents (Mattrey, Andre, Ultrasonic Enhancement of Myocardial Infarction with Perfluorcarbon Compounds in Dogs, Am J Cardiol 54: 206-210, 1984).

It has been seen that overall the gas-free contrast agents only have a low efficiency. The gas-containing preparations have the disadvantage of only a slight in-vivo stability. Furthermore the size of the gas bubbles can generally not be standardized. As a rule adequate contrast effects are not possible in the arterial vessel system after a peripheral veinous injection.

In EP A2 123 235 and 0 122 624 ultrasonic contrast agents are described which contain small gas bubbles and which pass through the pulmonary capillaries producing the desired contrast effect.

EP A2 0 224 934 describes ultrasonic contrast agents in the form of gas-filled gelatine or albumin hollow bodies. However the disadvantage here is the use of foreign-body albumens or denatured albumens belonging to the body and thus the associated risk of allergy.

With none of the ultrasonic contrast agents known up until now is it possible to represent the organs with sufficient signal intensity through selective concentration after an i.v. dose. Quantifications are therefore not possible at the present time.

The object of the present invention is to provide ultrasonic

contrast agents on the basis of microparticles which in addition to a determinable and reproduceable volume have a considerably longer service life than previously known, offer good compatibility without allergic potential and can be concentrated intracellularly in RES and thus also in the liver or spleen. The ultrasonic contrast agents comprise the microparticles in a physiologically acceptable carrier liquid.

This carrier liquid is preferably an aqueous medium in which the microparticles are suspended. The ultrasonic contrast agents can therefore be administered by injection, for instance.

Therefore one aspect of the present invention concerns an ultrasonic contrast agent comprising a physiologically acceptable carrier liquid, and microparticles which contain a gas and/or an organic fluid with a boiling point below 60°C, characterised in that said microparticles comprise one or more amyloses, or one or more synthetic biodegradable polymers.

Another aspect of the present invention concerns microparticles for use in ultrasonic contrast agents, said microparticles containing a gas and/or an organic fluid with a boiling point below 60°C, and said microparticles consisting of:
(a) one or more amyloses, or
(b) one or more synthetic biodegradable polymers.

The present invention concerns microparticles which consist of amylose, or a synthetic biodegradable polymer, and a gas and/or a fluid with a boiling point below 60°C.

Polyesters of α-, β-, γ-, or ε-hydroxy carbonic acids, polyalkyl-cyanoacrylates, polyamino acids, polyamides, polyacrylated saccharides or polyorthoesters are preferred as synthetic biodegradable polymers.

The following have proved particularly suitable:
Polylactic acid,
Poly-ε-caprolacton,
Copolymers of lactic acid and glycol acid or ε-caprolacton,
Polyhydroxybutyric acid,
Polyhydroxyvaleric acid,
Copolymers of hydroxybutyric and hydroxyvaleric acid,
Polymers of glutamic acid and/or lysine,
Polydioxanon,
Polymers or copolymers of amino acids and/or terephthalic acid, phthalic acid or sebacic acid,
Polyacryldextran,
Polyacryl starch,
Polyacrylamide,
Polyurethane,
Polyester,
Polyacetal,
Polyaminotriazol or
Polyalkylcyanoacrylate

Starch or starch derivatives can also be contained in the microparticles. Amyloses have proved particularly suitable since these starch derivatives have excellent water solubility and the ability to form inclusion compounds.

Amyloses which are particularly suitable are the cyclodextrines and their derivatives, by way of example α, β, and γ-cyclodextrin.

The microparticles contain gases and/or fluids with a boiling point below 60° in free or bonded form. The use of a gas-fluid mixture in the ultrasonic contrast agents is likewise possible.

Gases used can be for example air, nitrogen, inert gases, hydrogen, carbon dioxide, ammonia, oxygen, methane, ethane, propane, butane, ethylene or other hydrocarbons or their mixtures.

Preferred fluids which can be included are:
1,1-dichloroethylene,
2-methyl-2-butene,
isopropyl chloride,
2-methyl-1,3-butadiene,
2-butyne,
2-methyl-1-butene,
dibromodifluoromethane,
furan,
3-methyl-1-butene,
isopentane,
diethylether,
3,3-dimethyl-1-butyne,
dimethylaminoacetone,
propylene oxide,
N-ethylmethylamine,

bromomethane,
n-ethyldimethylamine,
methylene chloride,
pentane,
cyclopentane,
2,3-pentadiene,
cyclopentene
or mixtures thereof.

The microparticles can also contain advantageously substances with low steam pressures and/or low boiling points, in particular ethereal oils.

It is particularly advantageous to coat the microparticles with consist of amylose with a coating substance. The microparticles can thereby be encased in oils, fats and/or surface-active substances and suspended in an aqueous medium.

It is particularly advantageous to encase the microparticles which consist of amylose in a matrix, more particularly of a polymer structure.

The physiologic isotony can be set by the addition of osmotically active substances such as cooking salt, galactose, glucose, fructose, or mannitol (also known as mannite).

The invention also relates to a process for preparing the microparticles which consist of synthetic biodegradable polymers.

An advantageous process for preparing the microparticles, and ultimately the contrast agents according to the invention consists in dissolving a polymer or copolymer in one or more organic solvents which are not miscible with water, followed by the emulsification in water, possibly with the addition of a further solvent, and then filtering and if required drying the emulsion obtained.

A further process consists in dissolving a polymer or copolymer in one or more solvents which contain gas bubbles, after which it is precipitated or emulsified in water, if required with the addition of a further solvent or a further polymer, and then the suspension or emulsion which has been obtained is then filtered and if required dried. The freeze-drying process is also suitable as a finishing process.

The products obtained can advantageously be finely ground.

In the processes described, the solvents used can be for example furan, pentane, acetone, dioxan, ethyl acetate, xylol, methylene chloride, cyclohexane or hexane or solvent mixtures. Emulsifiers can also be added to the emulsion.

In a further variation of the manufacturing process instead of polymers monomers are used as the starting product from which the polymer is formed. With this process, a monomer is dissolved in one or more organic solvents and then emulsified in 5 - 30 parts water or 0.01 - 0.1 N hydrochloric acid, if required with the addition of emulsifiers or buffer substances at a temperature below the boiling point of the organic solvent, after which a 0.2% - 20% aqueous solution of a second monomer or if required the solution of a substance increasing the pH-value is added to this emulsion and dried if required.

In another method of operation a monomer is dissolved or dispersed in one or more fluids containing gas bubbles, if required with the addition of emulsifiers or buffer substances. If required a 0.2% - 20% solution of a second monomer or a substance increasing the pH-value in dissolved or gaseous form is added to this solution or dispersion and dried if required.

By way of example, terephthaloyl- or sebacoylchloride or cyanacrylic acid ester is used as a first monomer, L-lysine as the second monomer and for example 2-methyl-1,3-butadiene, dioxan, methylene chloride, toluene or cyclohexane is used as the organic solvent.

According to a further process, the ultrasonic contast agents are prepared by producing gas bubbles in a 0.5 -10% aqueous solution or dispersion of a monomer which contains if required additives such as emulsifiers (0.01 - 5%) or quasi emulsifiers (0.1 - 5%), and then by adding a cross-linking substance and/or a reaction starter.

The ultrasonic contrast agents described above can be used for both diagnostic and therapeutic processes.

The application of the agents is for example by injection.

The invention will be explained by the following examples:

EXAMPLE 1:

500 mg polylactide were dissolved in 4 ml furan and 0.6 ml cyclohexane and this solution was emulsified in 40 ml of a 0.1% solution of polyoxyethylene polyoxypropylene polymer with a molecular weight 12.000 (Pluronic® F 127), with the temperature being kept beneath 15°C during emulsifying. The temperature was then slowly raised to evaporate off the organic solvent. The resulting suspension was then freeze-dried.

EXAMPLE 2:

300 mg α-cyanoacrylic acid butyl ester were dissolved in 1 ml furan and this solution was emulsified in 10 ml 0.1 N hydro-

chloric acid which contained 1% polyoxyethylene polyoxypropylene polymer with a molecular weight 12.000 (Pluronic R F 127), with the temperature being kept beneath 15°C during emulsifying. At the end of polymerization the resulting suspension was freeze-dried.

EXAMPLE 3:

200 mg α-cyanoacrylic acid butyl ester were dissolved in 0.4 ml isoprene and emulsified in 30 ml 0.01 N hydrochloric acid which contained 1% polyoxyethylene polyoxypropylene polymer with a molecular weight 8.350 (Pluronic R F 68), with the temperature being kept beneath 10°C during emulsifying. At the end of the polymerization the suspension was neutralized with 0.1 N NaOH and isotonized with sodium chloride.

EXAMPLE 4:

400 mg α-cyanoacrylic acid butyl ester were dissolved in 0.4 ml methylene chloride and emulsified in 60 ml 0.01 N hydrochloric acid which contained 1% polyoxyethylene polyoxypropylene polymer with a molecular weight 12.000 (Pluronic R F 127), with the temperature being kept beneath 10°C during emulsifying. At the end of polymerization the suspension was neutralized with 0. 1 N soda lye and isotonized with sodium chloride.

EXAMPLE 5:

400 mg polycaprolactone were dissolved in 6 ml furan and 0.3 ml cyclohexane and emulsified in 60 ml 1% polyoxyethylene polyoxypropylene polymer with molecular weight 12.000 (Pluronic R F 127), with the temperature being kept beneath 15°C. The temperature was then slowly raised to evaporate off the organic solvent. The resulting suspension was then freeze-dried.

EXAMPLE 6:

400 mg terephthalic acid dichloride were dissolved in 2 ml furan and then emulsified in 50 ml 3% sodium carbonate solution which contained 0.1% polyoxyethylene polyoxypropylene polymer with a molecular weight 12.000 (Pluronic® F 127). After the additon of 60 mg L-lysine, dissolved in 5 ml 0.1% Pluronic F 127, the micro capsules were centrifuged and washed several times with 0.1% Pluronic F 127 solution. Before use the suspension was isotonized with sodium chloride.

EXAMPLE 7:

β-cyclodextrin-isopentane-inclusion compound:

100 ml saturated β-cyclodextrin solution (1.8%) were cooled to 10°C and mixed with 3 ml isopentane. The resulting difficultly soluble complex was precipitated with constant stirring in the ultrasonic bath. The deposit was obtained in crystalline form through freeze-drying and filtration. Isopentane content according to GC calculation : 0.26%

EXAMPLE 8:

β-Cyclodextrin-2-methyl-2-butene-inclusion compound:

100 ml saturated β-cyclodextrin solution (1.8%) were cooled to 10°C and mixed with 3 ml 2-methyl-2-butene. The resulting difficultly soluble complex was precipitated with constant stirring in the ultrasonic bath. The deposit was obtained in crystalline form through freeze-drying and filtering.

EXAMPLE 9:

β-Cyclodextrin-2-methyl-1-butene-inclusion compound:

100 ml saturated β-cyclodextrin solution (1.8%) were cooled to 10° and mixed with 3 ml 2-methyl-1-butene. The resulting difficultly soluble complex was precipitated with constant stirring in the ultrasonic bath. The deposit was obtained in crystalline form through freeze-drying and filtering. 2-methyl-1-butene content according to GC calculation: 0.82%

EXAMPLE 10:

β-cyclodextrin-isoprene-inclusion compound:

100 ml saturated β-cyclodextrin solution (1.8%) were cooled to 10°C and mixed with 3 ml isoprene. The resulting difficultly soluble complex was precipitated with constant stirring in the ultrasonic bath. The deposit was obtained in crystalline form through freeze-drying and filtering. Isoprene content according to GC calculation: 1.0%

EXAMPLE 11:

β-cyclodextrin-isopropylchloride-inclusion compound:

100 ml saturated β-cyclodextrin solution (1.8%) were cooled to 10°C and mixed with 3 ml isopropylchloride. The resulting difficultly soluble complex was precipitated with constant stirring in the ultrasonic bath. The deposit was obtained in crystalline form through freeze-drying and filtering. Isopropylchloride content according to GC calculation: 0.5%.

EXAMPLE 12:

β-cyclodextrin-isopentane-inclusion compound:

100 ml saturated β-cyclodextrin solution (1.8%) were cooled to 10°C and mixed with 3 ml isopentane. The resulting difficultly soluble complex was precipitated with constant stirring in the ultrasonic bath. The deposit was obtained in crystalline form through freeze-drying and filtering.

EXAMPLE 13:

Xenon/α-cyclodextrin-inclusion compound:

100 ml saturated α-cyclodextrin solution (about 12%) were incubated under 7 atmospheres xenon for 7 days at room temperature in a 200 cc autoclave. The crystalline adduct could be sucked off, washed with cold water and dried via calcium chloride in the exsiccator.

EXAMPLE 14:

Carbon dioxide/α-cyclodextrin-inclusion compound:

100 ml saturated α-cyclodextrin solution (about 12%) were incubated for 7 days at room temperature under 7 atmospheres carbon dioxide in a 200cc autoclave. The crystalline adduct could be drawn off, washed with cold water and dried via calcium chloride in the exsiccator.

EXAMPLE 15:

Isopentane/hydroxypropyl-β-cyclodextrin-inclusion compound: 15 ml 20% hydroxproply-β-cyclodextrin solution were mixed with 2 ml isopentane at 10°C, ultrasounded for 3 minutes in the ultrasonic bath and then incubated for 26 hours. The resulting complex remained in solution.

EXAMPLE 16:

Isoprene/hydroxypropyl- β-cyclodextrin-inclusion compound:

15 ml 20% hydroxypropyl -β-cyclodextrin solution were mixed with 2 ml isoprene at 10°C, ultrasounded for 3 minutes in the ultrasonic bath and then incubated for 26 hours. The resulting complex remained partly in solution and precipitated partly as a white deposit.

EXAMPLE 17:

Furan/hydroxypropyl-β-cyclodextrin-inclusion compound:

15 ml 20% hydroxypropyl-β-cyclodextrin solution were mixed with 2 ml furan at 10°C, ultrasounded for 3 minutes in the ultrasonic bath and then incubated for 26 hours. The resulting complex remained partly in solution and partly precipitated as a white deposit.

EXAMPLE 18:

Isopentane/α-cyclodextrin-inclusion compound:

20 ml saturated α-cyclodextrin solution were mixed with 1

ml isopentane and ultrasounded for 3 minutes in the ultrasonic bath. The resulting difficultly soluble complex was obtained through filtration and dried via calcium chloride.

EXAMPLE 19:

Isoprene/ α-cyclodextrin inclusion compound:

20 ml saturated α-CD-solution were mixed with 1 ml isoprene and ultrasounded for 3 minutes in the ultrasonic bath. The resulting difficultly soluble complex was obtained through filtration and dried via calcium chloride.

EXAMPLE 20:

Furan/ α-cyclodextrin-inclusion compound:

20 ml saturated α-cyclodextrin solution were mixed with 1 ml furan and ultrasounded for 3 minutes in the ultrasonic bath. The resulting difficultly soluble complex was obtained through filtration and dried via calcium chloride.

EXAMPLE 21:

4g eucalyptol was added dropwise to 100ml saturated α-cyclodextrin-solution (5°C) in an incubation chamber while being ultrasounded and was ultrasounded for a further 30 min. Thereafter the incubation chamber was shaken in a cooled, closed vessel for 48 hours. The resulting precipitate was filtered off, washed with cold ethanol, frozen in liquid nitrogen and freeze dried.

EXAMPLE 22:

100 ml saturated β-cyclodextrin-solution was ultrasounded with 2g Geraniol at 5°C for 4 hours and thereafter incubated for 24 hours at 5°C. The resulting precipitate was filtered off, washed with cold ethanol, frozen in liquid nitrogen and freeze dried.

The following applies to Examples 17 - 22:

The crystalline deposit was absorbed after cleaning in a suitable aqueous medium, preferably physiological cooking salt, glucose or Ringer solution and was then ready for injection.

The claims defining the invention are as follows:

1. An ultrasonic contrast agent comprising of physiologically acceptable carrier liquid, and microparticles which contain a gas and/or a organic fluid with a boiling point below 60°C, said microparticles consisting of:
(a) one or more amyloses, or
(b) one or more synthetic biodegradable polymers.

2. The ultrasonic contrast agent according to claim 1, comprising a physiologically acceptable carrier liquid, and microparticles which contain a gas and/or an organic fluid with a boiling below 60°C, characterised in that said microparticles comprise one or more amyloses.

3. The ultrasonic contrast agent according to claim 1, comprising a physiologically acceptable carrier liquid, and microparticles which contain a gas and/or an organic fluid with a boiling point below 60°C, characterised in that said microparticles comprise one or more synthetic biodegradable polymers.

4. The ultrasonic contrast agent according to claim 1 or claim 2, characterised in that the microparticles contain cyclodextrins or cyclodextrin derivatives as amylose.

5. Ultrasonic contrast agent according to claim 1 or claim 3, characterised in that the microparticles contain polyesters of α-, β-, γ- or ε-hydroxycarbonic acids, polyalkylcyanoacrylates, polyamino acids, polyamides, polyacrylated saccharides or polyorthoesters as the synthetic biodegradable polymers.

6. The ultrasonic contrast agent according to any one of

claims 1 to 5, characterised in that the microparticles contain as organic fluids with a boiling point below 60°C any one or more of:

1,1-dichloroethylene,
2-methyl-2-butene,
isopropylchloride,
2-methyl-1,3-butadiene,
2-butyne,
2-methyl-1-butene,
dibromodifluoromethane,
furan,
3-methyl-1-butene,
isopentane,
diethylether,
3,3-dimethyl-1-butyne,
dimethylamino acetone,
propylene oxide,
N-ethylmethylamine,
bromomethane,
N-ethyldimethyl amine,
methylene chloride,
pentane,
cyclopentane,
2,3-pentadiene,
cyclopentene,
including mixtures thereof.

7. Ultrasonic contrast agent according to any one or more of claims 1 to 5 characterised in that the microparticles contain as gases, any one or more of:
air,
inert gases,
nitrogen,
oxygen,

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carbon dioxide,
hydrogen,
ammonia,
ethylene,
methane,
ethane,
propane,
butane,
including mixtures thereof.

8. The ultrasonic contrast agent according to any one of claims 1 to 7 characterised in that the microparticles additionally contain ethereal oils.

9. The ultrasonic contrast agent according to claim 1 or claim 2 characterised in that the microparticles consisting of amyloses are coated with a hydrophobic covering substance which consists of oils, fats and/or surface-active substances, and are suspended in aqueous medium.

10. The ultrasonic contrast agent according to claim 1 or claim 2 characterised in that the microparticles consisting of amyloses are covered by a matrix.

11. The ultrasonic acid contrast agent according to claim 10, wherein the said matrix is of a polymer structure.

12. The ultrasonic contrast agent according to any one of claims 1 to 11 characterised in that physiological isotony is set by the addition of one or more osmotically active substances.

13. The ultrasonic contrast agent according to claim 12, wherein said osmotically active substance is selected from one or more of: cooking salt, mannitol, galactose, glucose, or fructose.

14. Microparticles for use in ultrasonic contrast agents, said microparticles containing a gas and/or an organic fluid with a boiling point below 60°C and said microparticles consisting of:
(a) one or more amyloses, or
(b) one or more synthetic biodegradable polymers.

15. The microparticles according to claim 14, characterised in that they consist of one or more amyloses.

16. The microparticles according to claim 14, characterised in that they consist of one or more synthetic biodegradable polymers.

17. The microparticles according to claim 15 characterised in that they consist of one or more cyclodextrins or cyclodextrin derivatives.

18. The microparticles according to claim 16, characterised in that they consist of one or more polyesters of α-, β-, γ- or ε-hydroxycarbonic acids, polyalkylcyanoacrylates, polyamino acids, polyamides, polyacrylated saccharides or polyorthoesters.

19. The microparticles according to any one of claims 14 to 18, characterised in that the microparticles contain as organic fluids with a boiling point below 60°C any one or more of:

1,1-dichloroethylene,
2-methyl-2-butene,
isopropylchloride,
2-methyl-1,3-butadiene,
2-butyne,
2-methyl-1-butene,

dibromodifluoromethane,
furan,
3-methyl-1-butene,
isopentane,
diethylether,
3,3-dimethyl-1-butyne,
dimethylamino acetone,
propylene oxide,
N-ethylmethylamine,
bromomethane,
N-ethyldimethyl amine,
methylene chloride,
pentane,
cyclopentane,
2,3-pentadiene,
cyclopentene,
including mixtures thereof.

20. The microparticles according to any one of claims 14 to 18, characterised in that the microparticles contain as gases, any one or more of:
air,
inert gases,
nitrogen,
oxygen,
carbon dioxide,
hydrogen,
ammonia,
ethylene,
methane,
ethane,
propane,
butane,
including mixtures thereof.

21. A process for the preparation of microparticles of synthetic biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16, or 18 characterised in that a polymer or copolymer is dissolved in one or more organic solvents which are not miscible with water, and is then emulsified in water, and the emulsion thus obtained is then filtered and dried.

22. A process for the preparation of microparticles of synthetic biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16 or 18 characterised in that a polymer or copolymer is dissolved in one or more solvents containing gas bubbles and is then precipitated or emulsified in water, the suspension or emulsion obtained is then filtered off and dried.

23. The process according to claim 21 or 22, characterised in that the furan, pentane, acetone, dioxan, ethylacetate, p-xylol, methylene chloride, cyclohexane or n-hexane or a solvent mixture consisting thereof is used as the solvent.

24. The process according to claim 21 or 22, characterised in that an emulsifier is added to the emulsion.

25. A process for the preparation of microparticles of synthetic, biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16 or 18 characterised in that a monomer is dissolved in one or more organic solvents and emulsified in 5 - 30 parts water or 0.01 - 0.1 N hyrochloric acid if required with the addition of emulsifiers or buffer substances at a temperature below boiling point of the organic solvent, and a 0.2 - 20% aqueous solution of a second monomer or if required the solution of a substance which raises the PH value is added to the emulsion and dried.

26. A process for the preparation of microparticles of synthetic biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16 or 18 characterised in that a monomer is dissolved or dispersed in one or more fluids containing gas bubbles, if required with the addition of emulsifiers and/or buffer substances, and to this solution or dispersion is added if required a 0.2 - 20% solution of a second monomer or a substance which raises the pH value in dissolved or gaseous form, followed by drying.

27. The process according to claim 25 or 26 characterised in that terephthaloyl- or sebacoyl chloride or cyanoacrylic acid ester is used as the first monomer, L-lysine as the second monomer and 2-methyl-1,3-butadiene, methylene chloride, toluene, dioxan or cyclohexane is used as the organic solvent.

28. A process for the preparation of microparticles of synthetic biodegradable polymers for use in ultrasonic contrast agents according to any one of claims 14, 16 or 18 characterised in that gas bubbles are produced in a 0.5 - 10% aqueous solution of a monomer which contains if required additives such as emulsifiers (0.01 - 5%) or quasi-emulsifiers (0.1-5%) and then a cross-linking substance and/or a reaction starter is/are added.

29. A method for the preparation of ultrasonic contrast agents, which comprise mixing together microparticles according to any one of claims 14 to 20 and a physiologically acceptable carrier liquid.

30. A method of ultrasound diagnostics characterised in that an ultrasonic contrast agent according to any one of claims 1 to 13 is administered to a subject for ultrasound diagnostics.

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31. Microparticles for use ultrasonic contrast agents, substantially as herein described with reference to any one of Examples 1 to 22.

32. Ultrasonic contrast agents comprising the microparticles according to claim 31 and a physiologically acceptable carrier liquid.

DATED this 14th day of January, 1993.

SCHERING AG
By Its Patent Attorneys
DAVIES COLLISON CAVE

0518e/DM

ABSTRACT

The invention relates to ultrasonic contrast agents consisting of microparticles which consist of amyloses and synthetic biodegradable polymers and a gas and/or a fluid with a boiling point below 60°C, process for the preparation thereof and their use as diagnostic and therapeutic agents.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DE 89/00069

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.4 A 61 K 49/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
Int.Cl.4	A 61 K ; A 61 B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP,A, 0123235 (SCHERING AG) 31 October 1984 see the whole document , in particular page 6,lines 19-21; page 7,lines7-11 . cited in the application	1,2,5-9,18
A	WO,A, 80/02365 (RASOR ASSOCIATES, INC .) 13 November 1980 see claims	1-18
A	FR,A, 2429616 (DOW CORNING CORP) 25 January 1980 see page 18, example 4	1
A	FR,A, 2496460 (CHINOIN GYOGYSZER- ES VEGYESZETI TERMEKEK GYARA RT) 25 June 1982 see pages 6-8, examples 1-5	1,6
A	DE,A, 3341001 (KRAUSE et al) 23 May 1985 see the whole document	1,3,10-17

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 May 1989 (12.05 89):	12 June 1989 (12.06.89)
International Searching Authority	**Signature of Authorized Officer**
European Patent Office	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. DE 8900069 SA 26583

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/06/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0123235	31-10-84	DE-A- 3313947	18-10-84
		AU-B- 569072	21-01-88
		AU-A- 2680684	18-10-84
		CA-A- 1232837	16-02-88
		DE-A- 3473829	13-10-88
		JP-A- 59205329	20-11-84
WO-A- 8002365	13-11-80	US-A- 4276885	07-07-81
		AU-A- 6053580	20-11-80
		CA-A- 1171952	31-07-84
		EP-A- 0028253	13-05-81
FR-A- 2429616	25-01-80	US-A- 4370160	25-01-83
		AU-B- 524895	07-10-82
		AU-A- 4790879	03-01-80
		CA-A- 1129373	10-08-82
		DE-A,C 2925305	03-01-80
		GB-A,B 2026513	06-02-80
		JP-A- 55005787	16-01-80
		JP-A- 60106837	12-06-85
FR-A- 2496460	25-06-82	JP-A- 57123114	31-07-82
DE-A- 3341001	23-05-85		

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen PCT/DE 89/00069

I. KLASSIFIKATION DES ANMELDUNGSGEGENSTANDS (bei mehreren Klassifikationssymbolen sind alle anzugeben)[6]

Nach der Internationalen Patentklassifikation (IPC) oder nach der nationalen Klassifikation und der IPC

Int Cl[4] A 61 K 49/00

II. RECHERCHIERTE SACHGEBIETE

Recherchierter Mindestprüfstoff[7]

Klassifikationssystem	Klassifikationssymbole
Int Cl[4]	A 61 K; A 61 B

Recherchierte nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Sachgebiete fallen[8]

III. EINSCHLÄGIGE VERÖFFENTLICHUNGEN[9]

Art*	Kennzeichnung der Veröffentlichung[11], soweit erforderlich unter Angabe der maßgeblichen Teile[12]	Betr. Anspruch Nr.[13]
A	EP, A, 0123235 (SCHERING AG) 31. Oktober 1984 siehe das ganze Dokument, insbesondere Seite 6, Zeilen 19-21; Seite 7, Zeilen 7-11 in der Anmeldung erwähnt --	1,2,5-9,18
A	WO, A, 80/02365 (RASOR ASSOCIATES, INC.) 13. November 1980 siehe die Patentansprüche --	1-18
A	FR, A, 2429616 (DOW CORNING CORP.) 25. Januar 1980 siehe Seite 18, Beispiel 4 --	1
A	FR, A, 2496460 (CHINOIN GYOGYSZER- ES VEGYESZETI TERMEKEK GYARA RT) 25. Juni 1982 siehe Seiten 6-8; Beispiele 1-5 -- ./.	1,6

* Besondere Kategorien von angegebenen Veröffentlichungen[10]:

"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist

"E" älteres Dokument, das jedoch erst am oder nach dem internationalen Anmeldedatum veröffentlicht worden ist

"L" Veröffentlichung, die geeignet ist, einen Prioritätsanspruch zweifelhaft erscheinen zu lassen, oder durch die das Veröffentlichungsdatum einer anderen im Recherchenbericht genannten Veröffentlichung belegt werden soll oder die aus einem anderen besonderen Grund angegeben ist (wie ausgeführt)

"O" Veröffentlichung, die sich auf eine mündliche Offenbarung, eine Benutzung, eine Ausstellung oder andere Maßnahmen bezieht

"P" Veröffentlichung, die vor dem internationalen Anmeldedatum, aber nach dem beanspruchten Prioritätsdatum veröffentlicht worden ist

"T" Spätere Veröffentlichung, die nach dem internationalen Anmeldedatum oder dem Prioritätsdatum veröffentlicht worden ist und mit der Anmeldung nicht kollidiert, sondern nur zum Verständnis des der Erfindung zugrundeliegenden Prinzips oder der ihr zugrundeliegenden Theorie angegeben ist

"X" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden

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"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

IV. BESCHEINIGUNG

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
12. Mai 1989	12 JUN 1989
Internationale Recherchenbehörde	**Unterschrift des bevollmächtigten Bediensteten**
Europäisches Patentamt	P.C.G. VAN DER PUTTEN

Internationales Aktenzeichen PCT/DE 89/00069

III. EINSCHLAGIGE VEROFFENTLICHUNGEN (Fortsetzung von Blatt 2)		
Art *	Kennzeichnung der Veroffentlichung, soweit erforderlich unter Angabe der maßgeblichen Teile	Betr. Anspruch Nr.
A	DE, A, 3341001 (KRAUSE et al.) 23. Mai 1985 siehe das ganze Dokument --------	1,3,10-17

ANHANG ZUM INTERNATIONALEN RECHERCHENBERICHT ÜBER DIE INTERNATIONALE PATENTANMELDUNG NR.

DE 8900069
SA 26583

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben.
Die Angaben über die Familienmitglieder entsprechen dem Stand der Datei des Europäischen Patentamts am 06/06/89
Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie	Datum der Veröffentlichung
EP-A- 0123235	31-10-84	DE-A- 3313947	18-10-84
		AU-B- 569072	21-01-88
		AU-A- 2680684	18-10-84
		CA-A- 1232837	16-02-88
		DE-A- 3473829	13-10-88
		JP-A- 59205329	20-11-84
WO-A- 8002365	13-11-80	US-A- 4276885	07-07-81
		AU-A- 6053580	20-11-80
		CA-A- 1171952	31-07-84
		EP-A- 0028253	13-05-81
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		AU-A- 4790879	03-01-80
		CA-A- 1129373	10-08-82
		DE-A,C 2925305	03-01-80
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		JP-A- 55005787	16-01-80
		JP-A- 60106837	12-06-85
FR-A- 2496460	25-06-82	JP-A- 57123114	31-07-82
DE-A- 3341001	23-05-85	Keine	

Für nähere Einzelheiten zu diesem Anhang : siehe Amtsblatt des Europäischen Patentamts, Nr.12/82